

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Previously presented): A method for producing a recombinant glycoprotein in a non-human eukaryotic host cell that expresses a glycosidase activity, the method comprising the step of diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure; wherein said method results in the production within the host cell of recombinant glycoproteins having N-glycans attached thereto comprising GlcNAcMan_xGlcNAc₂ core structures, wherein X is 3, 4, or 5.

Claim 2 (Previously presented): The method of claim 1, wherein the at least one glycosidase activity is expressed from a nucleic acid molecule introduced into the host cell.

Claim 3 (Previously presented): The method of claim 2, wherein the at least one glycosidase activity is a mannosidase activity.

Claim 4 (Previously presented): The method of claim 1, further comprising producing an N-glycan.

Claim 5 (Canceled)

Claim 6 (Previously presented): The method of claim 1, further comprising the step of expressing within the host cell one or more enzyme activities, selected from glycosidase and glycosyltransferase activities, to produce a GlcNAc₂Man₃GlcNAc₂ structure.

Claim 7 (Previously presented): The method of claim 6, wherein the one or more enzyme activities is selected from α -1,2 mannosidase, α -1,3 mannosidase and GnTII activities.

Claim 8 (Previously presented): The method of claim 1, wherein at least one diminished or depleted enzyme is selected from the group consisting of an enzyme having dolichyl-P-

Man:Man₅GlcNAc₂-PP-dolichyl alpha-1,3 mannosyltransferase activity; an enzyme having dolichyl-P-Man:Man₆GlcNAc₂-PP-dolichyl alpha-1,2 mannosyltransferase activity and an enzyme having dolichyl-P-Man:Man₇GlcNAc₂-PP-dolichyl alpha-1,6 mannosyltransferase activity.

Claim 9 (Previously presented): The method of claim 1, wherein the diminished or depleted enzyme has dolichyl-P-Man:Man₅GlcNAc₂-PP-dolichyl alpha-1,3 mannosyltransferase activity.

Claim 10 (Previously presented): The method of claim 1, wherein the enzyme is diminished or depleted by mutation of a host cell gene encoding the enzymatic activity.

Claim 11 (Previously presented): The method of claim 10, wherein the mutation is a partial or total deletion of a host cell gene encoding the enzymatic activity.

Claim 12 (Previously presented): The method of claim 1, wherein the attached N glycans have seven or fewer mannose residues.

Claim 13 (Canceled)

Claim 14 (Previously presented): The method of claim 1, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, GlcNAc, sialic acid, and fucose.

Claim 15 (Previously presented): The method of claim 1, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

Claim 16 (Previously presented): The method of claim 1, wherein the host is a lower eukaryotic cell.

Claim 17 (Previously presented): The method of claim 1, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*,

Aspergillus nidulans, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

Claim 18 (Withdrawn): The method of claim 1, wherein the host cell is further deficient in expression of initiating α -1,6 mannosyltransferase activity.

Claim 19 (Withdrawn): The method of claim 18, wherein the host cell is an *OCH1* mutant of *P. pastoris*.

Claim 20 (Withdrawn): The method of claim 1, wherein the host cell expresses GnTI and UDP-GlcNAc transporter activities.

Claim 21 (Withdrawn): The method of claim 1, wherein the host cell expresses a UDP- or GDP-specific diphosphatase activity.

Claim 22 (Withdrawn): The method of claim 1, further comprising the step of isolating the glycoprotein from the host.

Claim 23 (Withdrawn): The method of claim 22, further comprising the step of subjecting the isolated glycoprotein to at least one further glycosylation reaction in vitro, subsequent to its isolation from the host.

Claim 24 (Withdrawn): The method of claim 1, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more enzymes involved in the production of GlcNAcMan₃GlcNAc₂ or GlcNAc₂Man₃GlcNAc₂.

Claim 25 (Withdrawn): The method of claim 24, wherein at least one of the enzymes has mannosidase activity.

Claim 26 (Withdrawn): The method of claim 25, wherein the enzyme has an α -1,2-mannosidase activity and is derived from mouse, human, Lepidoptera, *Aspergillus nidulans*, *C. elegans*, *D. melanogaster*, or *Bacillus sp.*

Claim 27 (Withdrawn): The method of claim 25, wherein the enzyme has an α -1,3-mannosidase activity.

Claim 28 (Withdrawn): The method of claim 24, wherein at least one enzyme has glycosyltransferase activity.

Claim 29 (Withdrawn): The method of claim 28, wherein the glycosyltransferase activity is selected from the group consisting of GnTI and GnTII.

Claim 30 (Withdrawn): The method of claim 24, wherein at least one enzyme is localized by forming a fusion protein between a catalytic domain of the enzyme and a cellular targeting signal peptide.

Claim 31 (Withdrawn): The method of claim 30, wherein the fusion protein is encoded by at least one genetic construct formed by the in frame ligation of a DNA fragment encoding a cellular targeting signal peptide with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

Claim 32 (Withdrawn): The method of claim 31, wherein the encoded targeting signal peptide is derived from a member of the group consisting of mannosyltransferases, diphosphatases, proteases, GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, FT, and ST.

Claim 33 (Withdrawn): The method of claim 31, wherein the catalytic domain encodes a glycosidase or glycosyltransferase that is derived from a member of the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, Fucosyltransferase and ST.

Claim 34 (Withdrawn): The method of claim 31, wherein the nucleic acid molecule encodes one or more enzymes selected from the group consisting of UDP-GlcNAc transferase, UDP-galactosyltransferase, GDP-fucosyltransferase, CMP-sialyltransferase, UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, CMP-sialic acid transporter, and nucleotide diphosphatases.

Claim 35 (Withdrawn): The method of claim 31, wherein the host expresses GnTI and UDP-GlcNAc transporter activities.

Claim 36 (Withdrawn): The method of claim 31, wherein the host expresses a UDP- or GDP-specific diphosphatase activity.

Claim 37 (Withdrawn): The method of claim 1, further comprising the step of introducing into a host that is deficient in dolichyl-P-Man:Man₅GlcNAc₂-PP-dolichyl alpha-1,3 mannosyltransferase activity a nucleic acid molecule encoding one or more enzymes for production of the N-glycans comprising GlcNAcMan_xGlcNAc₂ core structures.

Claim 38 (Withdrawn): The method of claim 1, further comprising the step of introducing into a host that is deficient in dolichyl-P-Man:Man₆GlcNAc₂-PP-dolichyl alpha-1,2 mannosyltransferase or dolichyl-P-Man:Man₇GlcNAc₂-PP-dolichyl alpha-1,6 mannosyltransferase activity a nucleic acid molecule encoding one or more enzymes for production of the N-glycans comprising GlcNAcMan_xGlcNAc₂ core structures.

Claim 39 (Withdrawn): The method of claim 37 or 38, wherein the nucleic acid molecule encodes at least one enzyme selected from the group consisting of an α -1,2 mannosidase, UDP GlcNAc transporter and GnT1.

Claim 40 (Withdrawn): The method of claim 39, further comprising the step of introducing into the deficient host cell a nucleic acid molecule encoding an α -1,3 or an α -1,2/ α -1,3 mannosidase activity for the conversion of the GlcNAc1Man₄GlcNAc₂ structure to a GlcNAcMan₃GlcNAc₂ structure.

Claim 41 (Withdrawn): The method of claim 1, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more enzymes for production of a GlcNAc₂Man₃GlcNAc₂ carbohydrate structure.

Claim 42 (Withdrawn): The method of claim 41, wherein at least one enzyme is GnTII.

Claim 43 (Withdrawn): The method of claim 1, further comprising the step of introducing into the host cell at least one nucleic acid molecule encoding at least one mammalian glycosylation enzyme selected from the group consisting of a glycosyltransferase,

fucosyltransferase, galactosyltransferase, N-acetylgalactosaminyltransferase, N-acetylglucosaminyltransferase and sulfotransferase.

Claim 44 (Withdrawn): The method of claim 1, comprising the step of transforming host cells with a DNA library to produce a genetically mixed cell population expressing at least one glycosylation enzyme derived from the library, wherein the library comprises at least two different genetic constructs, at least one of which comprises a DNA fragment encoding a cellular targeting signal peptide ligated in-frame with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

Claim 45 (Withdrawn): A host cell produced by the method of claim 1 or 44.

Claim 46 (Previously presented): A human-like glycoprotein produced by the method of claim 1 or 44.

Claim 47 (Withdrawn): A nucleic acid molecule comprising or consisting of at least forty-five consecutive nucleotide residues of Fig. 6 (*P. pastoris* *ALG 3* gene).

Claim 48 (Withdrawn): A vector comprising a nucleic acid molecule of claim 47.

Claim 49 (Withdrawn): A host cell comprising a nucleic acid molecule of claim 47.

Claim 50 (Withdrawn): A *P. pastoris* cell in which the sequences of Fig. 6 (*P. pastoris* *ALG 3* gene), are mutated whereby the glycosylation pattern of the cell is altered.

Claim 51 (Withdrawn): A method to enhance the degree of glucosylation of lipid-linked oligosaccharides comprising the step of increasing alpha-1,3 glucosyltransferase activity in a host cell.

Claim 52 (Withdrawn): A method to enhance the degree of glucosylation of lipid-linked oligosaccharides comprising decreasing the substrate specificity of oligosaccharyl transferase activity in a host cell.

Claim 53 (Withdrawn): A method for producing in a non-mammalian host cell an immunoglobulin polypeptide having an N-glycan comprising a bisecting GlcNAc, the method comprising the step of expressing in the host cell a GnTIII activity.

Claim 54 (Withdrawn): A non-mammalian host cell that produces an immunoglobulin having an N-glycan comprising a bisecting GlcNAc.

Claim 55 (Withdrawn): An immunoglobulin produced by the host cell of claim 54.

Claim 56 (Withdrawn): A method for producing in a non-human host cell a polypeptide having an N-glycan comprising a bisecting GlcNAc, the method comprising the step of expressing in the host cell a GnTIII activity.

Claim 57 (Withdrawn): A non-human host cell that produces a polypeptide having an N-glycan comprising a bisecting GlcNAc.

Claim 58 (Withdrawn): A polypeptide produced by the host cell of claim 57.

Claim 59 (Previously presented): A method for producing a human-like glycoprotein in a non-human eukaryotic host cell comprising the step of diminishing or depleting from the host cell an *alg* gene activity and introducing into the host cell at least one glycosidase activity.

Claim 60 (Withdrawn) : A method for producing a human-like glycoprotein having an N-glycan comprising at least two GlcNAcs attached to a trimannose core.

Claim 61 (Withdrawn): The method of claim 1, wherein the host cell further expresses a GnTIII activity.

Claim 62 (Withdrawn): The method of claim 1, wherein the recombinant glycoprotein is an immunoglobulin.

Claim 63 (Withdrawn) The method of claim 61 or 62, wherein the recombinant glycoprotein is an immunoglobulin comprising a bisecting GlcNAc.

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Claim 64 (Withdrawn): The method of claim 37 or 38, wherein the GlcNAcMan_xGlcNAc₂ core structures comprise predominantly GlcNAcMan₄GlcNAc₂.

Claim 65 (Withdrawn): The method of claim 37 or 38, wherein the GlcNAcMan_xGlcNAc₂ core structures comprise predominantly GlcNAcMan₃GlcNAc₂.